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Apolipoprotein E ε4 and testosterone interact in the risk of Alzheimer's disease in men

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SUMMARY

The apolipoprotein E ε4 allele (*APOE*ε4) is a well-established risk factor for Alzheimer's disease (AD), but the mechanisms for this association are not well understood. In addition, other risk and protecting factors are needed to explain the causality of the disease. Sex steroid hormones, such as estradiol and testosterone, are thought to exert protective mechanisms in the brain (Lee and McEwen, 2001). Lower levels of total estradiol and total testosterone in men with AD have been found (Hogervorst *et al.*, 2001; Rasmuson *et al*., 2002). The current study examined relations between *APOE*ε4 and levels of total testosterone and total estradiol in the risk of AD in men.

KEY WORDS — Alzheimer's disease; testosterone; apolipoprotein E ε4 allele; risk

SUBJECTS, METHODS AND RESULTS

We examined 116 male Caucasians from the Oxford Project To Investigate Memory and Ageing (OPTIMA). Fifty-one were autopsy confirmed CERAD AD cases (mean age at episode: 75.3 years, range: 58.8–89.5 years), 10 were diagnosed 'probable AD' by NINCDS/ADRDA criteria (69.8 years, range: 57.4–88.8 years) and 55 were without cognitive impairment and with CAMCOG scores ≥ 80 (73.6 years, range: 39.9–94.7 years). All had given their informed consent prior to the study (Clarke *et al.*, 1998).

We analysed total testosterone using a competitive enzyme immunoassay (Bayer®, Bayer Cooperation, Tarrytown, NY, USA) in non-fasting blood serum samples that had been stored at −70°C. Serum had been collected between 10 and 12 am. For total estradiol, duplicate serum samples were extracted with ether. Estradiol was then assessed by radioimmunoassay using a highly specific rabbit antiserum. SHBG levels were investigated using an immuno-enzymometric assay (IEMA). Subjects were genotyped by standard PCR methods for *APOE* and for the butyrylcholinesterase K variant.

Using a logistic regression model with age and SHBG as co-variables, low testosterone (odds ratio (OR) = 0.86, 95% confidence intervals (CI) 0.75–0.99) and the *APOE*ε4 × testosterone interaction (OR = 1.28, 95% CI = 1.07–1.54) were significantly associated with AD, which suggested that the presence of the *APOE*ε4 allele modified the risk of AD associated with low testosterone levels. Entering *APOE*ε4 by itself as a risk factor for AD, gave an OR of 7.72 (95% CI = 3.63 to 16.48, $p < 0.01$). In a model where estradiol replaced testosterone, neither estradiol alone nor the estradiol × *APOE*ε4 interaction was a significant predictor of AD.

Table 1 presents the results of analyses stratified by diagnosis. The two main results were: first, testosterone levels were lower in *APOE*ε4-positive controls than in those without *APOE*ε4; second, in men without *APOE*ε4, testosterone levels were lower in AD than in controls. One of the lowest testosterone levels

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Table 1. Sex steroid levels in AD and in controls, by *APOE*ε4 status

Steroid	Subgroup (*n*)	*APOE*ε4 status (*n*)	Mean[a]	*p* (*t*-test)[b]
Testosterone				
	AD (61)	With (44)	13.9 (±5.1)	NS
		Without (17)	15.0 (±4.8)[c]	
	Controls (53)	With (12)	11.3 (±7.8)	<0.001
		Without (41)	19.1 (±5.4)[c]	
Estradiol				
	AD (60)	With (43)	69.8 (±28.7)	0.003
		Without (17)	94.9 (±28.7)	
	Controls (55)	With (12)	70.6 (±42.4)	0.003
		Without (43)	107.8 (±34.9)	

AD, Alzheimer's disease; *APOE*ε4, apolipoprotein E ε4; NS, not significant.
[a]Testosterone levels are in nmol/l and estradiol in pmol/l.
[b]All subgroups were normally distributed; equal variances could be and were assumed in all cases.
[c]Testosterone, *APOE*ε4-negative AD cases *vs* *APOE*ε4-negative controls: $p = 0.009$ (*t*-test).

(1.6 nmol/l) was in the only control who was an *APOE*ε4 homozygote. With estradiol, levels were lower in *APOE*ε4 carriers, both in AD cases and in controls.

We also examined two other alleles, apolipoprotein E ε2 (*APOE*ε2) and the butyrylcholinesterase K variant (*BCHE*-K). No significant association was found between either allele and either steroid in any analysis. Only a weak tendency was seen for *APOE*ε2-positive controls to have higher steroid levels than those without *APOE*ε2 (e.g. for testosterone: 20.2 nmol/l ($n = 11$) *vs* 16.5 nmol/l ($n = 42$), $p = 0.1$, *t*-test).

COMMENT

Table 1 illustrates two important results. First, *APOE*ε4 is associated with lower testosterone levels in men, but only significantly so in controls. Second, testosterone levels are higher in controls than in AD, but only in men without *APOE*ε4. These results are open to various interpretations. One is that *APOE*ε4, among other factors, lowers testosterone in male controls and low testosterone, whether or not due to *APOE*ε4 status, contributes to the onset of AD. Another interpretation is that *APOE*ε4 lowers testosterone in controls and that AD results in a lowering of testosterone levels for other reasons. This study cannot distinguish between these interpretations. Prospective studies are needed to resolve these issues.

Low testosterone is potentially a modifiable risk factor. At present, no long-term studies have investigated the possibly protective effects of testosterone against the development of AD. Short-term studies with testosterone replacement therapy in non-demented men have given mixed results (Wolf *et al.*, 1999). A possible explanation could be that, since *APOE*ε4-positive controls had lower levels in the present study than those without the allele, perhaps only *APOE*ε4-positive men would profit from testosterone replacement therapy. Future, long-term studies should investigate the possibly protective effect of testosterone replacement therapy in *APOE*ε4 carriers who are at risk of AD.

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